Art. XXXVIII.—*Protozoa Parasitic in the Large Intestine of Australian Frogs, Part I.*

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(With Plates XCIV.–XCV.).

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The following investigation was carried out at the suggestion of Professor Spencer, whom I wish to thank for his help and advice. It is an endeavour to record the different protozoa parasitic in the large intestine of our Australian Frogs, and to see how they compare with those in European species. So far I have obtained for examination only five species—viz., *Hyla aurea*, *H. ewingii*, *H. peronii*, *Limnodynastes dorsalis* and *L. tasmaniensis*, but I hope before long to have other species for investigation.

In examining the contents of the large intestine I have followed largely the methods employed by Dobell (4, 5, 6, 7, 8) in his papers on the protozoa parasitic in European forms. The contents have been kept in a 0.5 per cent. salt solution containing egg albumen and I have thus been able to keep the protozoa alive for several days. For fixing I have used chiefly Schaudinn's corrosive sublimate (hot), and for staining Heidenhain's iron haematoxylin with or without eosin, picro-carmine, acetic acid alum carmine, and acid haematoxylin.

I will now proceed to enumerate the different forms of protozoa present.

A. Ciliata.

Two forms that I have always found to be very abundant in the intestine are *Nyctotherus* and *Opalina*. These are present in varying proportions in the different frogs, but gene-

rally speaking the *Opalinae* are in greater numbers. Only very rarely are they ever completely absent, and then it seems to be owing to the lack of food material in the intestine. Taking the *Nyctotherus* first, the following is an account of the species represented in the large intestine.

Nyctotherus cordiformis, Ehrenberg.

This measures on an average $213\mu \times 128\mu$, but larger and smaller specimens are also present. In three of the species of frog examined—viz.: *H. aurea, H. ewingii* and *H. tasmaniensis* a very large form of Nyctotherus is present, sometimes, but rarely, in great numbers. This I take to be the same species, but of an abnormally large size, measuring on an average 398 μ long and 255 μ broad, and appearing quite giant in size as compared with the others (Fig. 4). Figure 5 shows one of these with a small individual inside it which thus enables us to see how very much they differ in size. I might here mention that individuals of extreme size as compared with the normal have been observed in other groups of the Protozoa. Thus Dobell (4) records the presence in *Trichomastix serpentis* of giant forms when he says: "An individual, instead of dividing when it reached a certain size, continued to grow. In this way giant individuals arose which reached the enormous length of 30 μ, i.e., about twice the normal length." These, however, were involution forms which were produced through overfeeding, whilst my remarkably large forms are present in the intestine living under normal conditions. They may, however, have been produced in much the same way by over-feeding.

A peculiar structure visible in some of the normal specimens stained in Heidenhain's iron haematoxylin is a backwardly directed flagellum (Fig. 2) running down from the posterior end of the oral groove and then curving forwards towards the entrance to the cytopharynx. This is very definite in these preparations but does not show up with other stains. I can find no reference concerning it anywhere, unless I regard Saville-Kent's long, stiff and outwardly projecting seta of this

species as corresponding to it. He figures this in his "Manual of the Infusoria" (vol. ii., plate xxix., fig. 4) from Stein, and describes the widened entrance or vestibulum to the pharynx as "bearing on its lower edge a single long, stiff seta." This is figured also in Bronn's Klassen und Ordnungen des Thier-Reichs (I. 3. Protozoa, plate 66, figs. 5a and 5b). Some six or eight individuals show this very clearly, though in most of them it is smaller than the one figured. With iron-haematoxylin alone, the nucleus, oral groove and flagellum appear black (Fig. 2), but when double stained with eosin they are not so sharply marked, the flagellum rarely being visible. Double-stained with orange G, it is clearer than with eosin.

In Fig. 1 the position of the macro-nucleus is shown, but I have never once been able to distinguish a micro-nucleus, and after having used all the usual stains I can definitely state that there has been none present. Occasionally the animal at first sight appeared to have a small nucleus in the position in which one would expect the micro-nucleus to be, but on further examination it has proved to be some foreign body lying on the outside of the animal. In Calkins' Protozoa (page 188) we read: "The functions of the two nuclei are supposed to be respectively vegetative and reproductive (Butschli), but this distinction is perhaps too sweeping. Julin ('93) held that the macro-nucleus stands not only for nutrition, movement, sensation and regeneration, but for asexual division as well, in fact is a 'somatic nucleus,' while the micro-nucleus functions only as a sexual nucleus." On this ground therefore it might be possible to account for the absence of the micro-nucleus, for the only form of reproduction I have observed as yet is the simple transverse division (Fig. 3), which function we thus see may be carried out in the absence of the micro-nucleus.

Among the *Opalinae* there are three forms represented, one of these the commonest, i.e., the one most often present and in greatest numbers, is *O. intestinalis* (Stein). A detailed description of this well-known form is unnecessary, but the chief points I have noted are as follows:—It measures from 107 μ to 214 μ in length and is ciliated equally all over, the cilia being very large. The nuclei are spherical and placed in the

anterior third of the body. They do not show the thread-like structure connecting them which is shown in Metcalf's figure (12). The chromatin material is gathered into masses around the periphery of the nucleus. Ectosarc and endosarc are clearly distinguished, but I have not been able to make out any excretory organs (Fig. 6). Very small forms occur along with these, and I think these represent the younger individuals of the same species, for they seem to graduate up to the adults in size. This species I have found in *H. aurea*, *H. ewingii* and *Limnodynastes dorsalis*.

Degeneration in these forms took place soon after they were removed from the host. First of all the body gradually altered its shape by the swelling up of the ectosarc and became more and more spherical. After a while the ectosarc and endosarc merged into one another and became indistinguishable. The cilia gradually ceased moving and ultimately the body began to disintegrate and was attacked by countless bacteria.

Opalalina binucleata, n. sp.

This is found in great numbers in *Limnodynastes dorsalis*, and on one occasion I met with it in *Limnodynastes tasmaniensis*. It is a broad, flat form with two nuclei, and is ciliated equally all over its surface, the cilia being arranged in longitudinal rows as in other Opalinae. It is broader and more bluntly pointed at the posterior end than at the anterior (Fig. 7) and moves along with the anterior end foremost. Its usual position when swimming along is on either flat surface, but as it proceeds it occasionally rolls over from side to side. The average length is 157 μ and the average breadth 100 μ, but larger and smaller individuals have been met with. When the animal turns over and presents itself edge on, it is seen to be very thin as compared with its breadth (Fig. 8), and in section would appear flat and oval. Metcalf (12) divides Opalinae into the following groups:—

(1) Species with two nuclei, bodies circular in cross section.

(2) Species with many nuclei, body circular or broadly oval in cross section.

(3) Species with many nuclei, body flattened.

To these we may now add—

(4) Species with two nuclei, body flattened. The nuclei measure 20 μ across, and are circular in outline, and placed obliquely behind each other. The chromatin material is scattered about in masses and is not arranged in any definite order. There is no differentiation into ectosarc and endosarc visible from a general surface view, and the protoplasm appears vacuolated. During movement the posterior portion of the body shows a rigid or rucked appearance as indicated in Fig. 9, so that it seems to be contracted towards this end, and in this way it moves along.

Opalina hylarum, n. sp.

Occurs in *Hyla aurea* only, and is distinguished from all the other binucleated forms which are circular in cross section by its enormous size. It measures on an average about 420 μ, but some individuals measuring as much as 572 μ have been met with. The average breadth is 70 μ. The body is elongately oval with a rounded anterior end and a slightly rounded posterior extremity, i.e., it does not taper to a point posteriorly. The protoplasm is granular, and ectosarc and endosarc are clearly distinguishable right to the posterior end. A very well-marked feature of this species is the position of the nuclei, for they are placed very far apart, the hinder one being in the posterior half of the body. (Fig. 10.) The chromatin material is gathered into masses arranged around the periphery of the nucleus. This is well shown in the transverse section represented in Fig. 11. The body is ciliated round its entire surface, the cilia at the anterior end being slightly larger than those towards the posterior end, but there is no posterior portion devoid of cilia (Fig. 15).

Some individuals showed only a single nucleus, in different stages of division, but these are the results of recent longitudinal division. In Figs. 12 to 14 the outlines of three specimens are shown with the positions of the nuclei indicated. In Figs. 12 and 13 the daughter nuclei have not yet separated,

while in Fig. 14 division of the nucleus is completed and the nuclei have taken up their adult position.

In the posterior portion of the body excretory organs are present in the form of a great number of vacuoles, forming quite a network and extending from about the middle of the body to the posterior extremity. (Figs. 10 and 15.)

B. Flagellata.

The commonest flagellate form present in the intestine is one which I take to be identical with the *Euglenoidina* from *Rana temporaria* and *Bufo vulgaris* described by Dobell, viz., *Copromonas subtilis*. It is found in countless numbers in cultures that have been standing for some days, and is present in all the species of frog examined. On account of its small size I have not been able to make out all the minute details of structure, nor have I traced through the different stages of its life history, but its general form and movements agree so closely with *Copromonas subtilis* that I think we may say it is identical (Fig. 16). A few individuals were observed undergoing longitudinal division (Fig. 17), and also some in conjugation (Fig. 18).

Occurring along with these but in far smaller numbers is a similar form which is more rectangular in outline and slightly longer. I have not succeeded in obtaining any of these mounted on the slide, but in the living state they appear very similar in structure to the oval forms.

The other flagellate forms present in the large intestine are the trichomonads. These I have found in large numbers in four out of the five species of frog examined, there being none in *Hyla ewingii*. This need not mean, however, that they are absent in this species altogether, for I may have missed them in examining the culture, or they may have been absent only from the particular individuals under inspection. Dobell (7) says as regards the trichomonads: "It has hitherto been universally supposed that but one trichomonad occurs in frogs, namely *Trichomonas batrachorum*, Perty. There are, however, in reality two, a *Trichomonas* and a *Trichomastix*." The latter is represented by *Trichomastix batrachorum*, Dobell, which he goes on to describe.

Both these forms I can say are present in the frogs I have examined, occurring sometimes together, sometimes separately, though it has often been difficult to distinguish between them, and at times quite impossible. The main difference between the two is the presence of an undulating membrane in *Trichomonas* in place of the posterior flagellum of *Trichomastix*, otherwise a description of the one would hold good for the other also. The body has an average length of about 12 μ and is oval, or spindle-shaped. The protoplasm is clear, excepting for the food granules at the posterior end and the nucleus at the anterior (Figs. 19 to 22). The axostyle is sometimes visible as a clear rod running through the body and projecting at the posterior end, giving the body a spindle-shaped appearance; at other times it seems absent altogether and the body then assumes an oval shape. Its form changes, however, very rapidly and becomes amoeboid, and it is in this amoeboid stage in particular that the two trichomonads appear so alike, and in fact are quite indistinguishable from one another. A remarkable form of movement was shown by one individual which appeared to be in a dying condition. A peculiar elongated process was forced in and out by sudden contractions and expansions. The whole body was amoeboid and the process looked as if it might have been either the undulating membrane drawn out from the body, if it was a *Trichomonas*, or the flagella fused to the body and then extended, if a *Trichomastix* (Fig. 23).

A somewhat similar movement was observed by Dobell in *Trichomastix serpentis*.

In the fresh forms the axostyle is sometimes swollen out near its posterior end as represented in Fig. 22, and this may appear to become confluent with the body when the central portion of the axostyle is disappearing.

LITERATURE.

(1) Bezzenberger. —"Uber Infusorien aus asiatischen Anuren." Arch. f. Protistenk. Band 3, 1904.

(2) Blochmann, F.—"Bemerkungen uber einige Flagellaten." Zeitschr. f. wiss. Zool., Band 40, 1884.

(3) Butschli, O.—"Protozoa" in Bronn's Thierreich ii. and iii., 1883-1889.

(4) Dobell, C. C.—"Trichomastix serpentis, n.sp." Quart. Journ. Microsc. Sci., vol. 51, 1907.

(5) Dobell, C. C.—"Notes on some Parasitic Protists." Quart. Jour. Microsc. Sci., vol. 52, 1908.

(6) Dobell, C. C.—"The Structure and Life History of Copromonas subtilis, nov. gen., nov. spec." Quar. Jour. Micr. Sci., vol. 52, 1908.

(7) Dobell, C. C.—"Researches on the Intestinal Protozoa of Frogs and Toads." Quar. Jour. Microsc. Sci., vol. 53, 1909.

(8) Dobell, C. C.—"On the Intestinal Protozoan Parasites of Frogs and Toads." Proc. Cambridge Phil. Soc., xiv. (iv.), 1908.

(9) Doflein, F.—Lehrbuch der Protozoenkunde, 1909.

(10) Leger et Duboscq.—"Notes sur les Infusoires endoparasites." Arch. de Zool. Expe. et Gen., 1904.

(11) Metcalf, M. M.—"The Excretory Organs of Opalina." Arch. f. Protistenk. Band x., 1907.

(12) Metcalf, M. M.—"Opalina," Oberlin Coll. Lab. Bulletin. Number xv., reprint from Arch. f. Protistenk. Band xiii., Heft. 3, 1909.

(13) Saville-Kent.—A Manual of the Infusoria.

(14) Zeller, E.—"Untersuchungen uber die Fortpflanzung und Entwicklung der in unseren Batrachiern schmarotzenden Opalinen." Zeitschr. f. wiss. Zool., Band xxix., 1877.

EXPLANATION OF PLATES XCIV.-XCV.

In all figures

A.N. = Anterior nucleus.
ECTO. = Ectosarc.
ENDO. = Endosarc.
EXCR. = Excretory Organs.
M.N. = Macro-nucleus.
P.N. = Posterior nucleus.

Fig. 1.—*Nyctotherus cordiformis*, showing macro-nucleus.

Fig. 2.—*Same*, stained with Heidenhain's iron haematoxylin, showing peculiar backwardly projecting flagellum at end of oral groove. Drawn with the camera lucida.

Fig. 3.—*Same*, undergoing transverse division, showing macro-nucleus dividing. Drawn with the camera lucida.

Fig. 4.—*Same*, drawn with the camera lucida to show the relative sizes of the giant form, Fig. 4a, and the normal form, 4b.

Fig. 5.—Outline of *same*, showing small form inside giant form.

Fig. 6.—Diagram of *Opalina intestinalis*, showing cilia all round, ectosarc and endosarc, and rounded nuclei.

Fig. 7.—Diagram of *O. binucleata*, showing anterior and posterior nuclei, cilia, and vacuolated protoplasm. Drawn with the camera lucida.

Fig. 8.—Drawing of a living *O. binucleata* as it appears when swimming edge on.

Fig. 9.—Drawing of *same*, showing ribbed appearance at the posterior end.

Fig. 10.—Drawing of *O. hylarum*, showing position of nuclei, ectosarc and endosarc.

Fig. 11.—Transverse section of *same*, showing arrangement of chromatin material in the nucleus.

Figs. 12 to 14.—Outlines of three specimens of *same*, showing different positions of nuclei. In Figs. 12 and 13 the nucleus is dividing; in Fig. 14 nuclear division is completed and nuclei have taken up their adult positions.

Fig. 15.—Drawing of *same*, showing excretory organs.

Figs. 16 to 18.—Drawing of *Copromonas subtilis*. In Fig. 16 the adult form is shown; in Fig. 17 longitudinal division; in Fig. 18 conjugation.

Figs. 19 to 22.—Trichomonads. Figs. 19 and 20 represent *Trichomonas batrachorum*, and Figs. 21 and 22 represent *Trichomastix batrachorum*.

Fig. 23.—Diagram to show peculiar movements of a dying Trichomonad.